A STUDY OF THE RATE OF REGENERATION OF THE ARMS IN THE BRITTLE-STAR, OPHIOGLYPHA LACERTOSA.

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Two interesting internal factors regulating the rate of regeneration of the arms in the brittle-star, *Ophioglypha lacertosa*, were discovered in the course of a study of the problem at the Naples Zoölogical Station during the past winter (1902–03). As some months must elapse before a full discussion of the results can be published it has been thought advisable to give the general data in a preliminary paper. This seems especially desirable at the present time because of the high interest taken in the experimental evidences of a far-reaching correlation between the parts of the individual in both animals and plants.

The experiments to be described give data which show that the rate of regeneration of the arms varies on the one hand with the size of the animal and on the other with the number of removed arms. The first-mentioned correlation gives a maximum rate of regeneration for the medium sized individuals with a pronounced decrease for the smaller as well as for the larger ones. The second correlation, with one exception to be mentioned, gives us an increase in the rate of regeneration of an arm as we pass from the cases with a smaller to those with a greater number of removed arms. The series with all five arms missing is excepted in the statement because the animals in this lot in every instance died or showed evidences of decay before the completion of the experiment.

Method. — Forty-five perfect specimens were divided into five equal groups of nine each, care being taken to distribute them in such a way as to make the sets approximately equivalent as regards size of individuals. The operation consisted in the removal of one or more arms by a transverse cut at the disk level. In the first series one arm was removed, in the second two contiguous arms, in the third three contiguous arms, in the fourth four, and in the fifth five arms. The animals were kept in ten "battery" jars, two for each series, and *were not fed during the whole period*

of the experiment. Measurements of the lengths of the regenerating arms were taken 22, 33 and 46 days after the operation. As stated above the specimens in the series where all five arms were removed did not retain their vitality for a sufficient length of time to allow of comparison with the others and they will therefore be excluded from the following tabulation.

As the rate of regeneration varies with the size of the animal as well as with the number of removed arms the proper relations can best be represented by means of curves. The figure accompanying this paper gives the average of Series I. and II. (those with respectively one and two arms removed) in one curve and of Series III. and IV. (those with respectively three and four arms removed) in the other. The same arrangement is followed for each of the three measurements taken respectively 22 days, 33 days and 46 days after the operation. This brings out the desired relations more clearly than would have been possible if all the individual data had been included. In the figure the abscissæ give the size of the animal as represented by the disk diameter in millimeters. The ordinates give the length of the regenerating arm or arms, also in millimeters. In the series where more than one arm was operated on the regeneration length as given is an average of all the regenerating arms of the individual. As the individual disk diameters are not exactly equivalent in the different series it was found convenient in taking the averages for the combination curves to use arbitrarily disk diameters equal to whole millimeters as the points for comparison. The curves in the figure are therefore constructed on this basis. The unbroken line in each case gives the average of Series I. and II. (one and two arms removed) and the broken line of Series III. and IV. (three and four arms removed).

Statement of Data and Discussion. — The curves show very distinctly the correlation between the rate of regeneration and the size of the animal on the one hand and the number of removed arms on the other.

1. Taking up first the size correlation we find that, starting with the smaller individuals, as we advance toward the larger ones there is a general increase up to a maximum at a diameter of 12 to 15 mm. This is most striking in the two later meas-

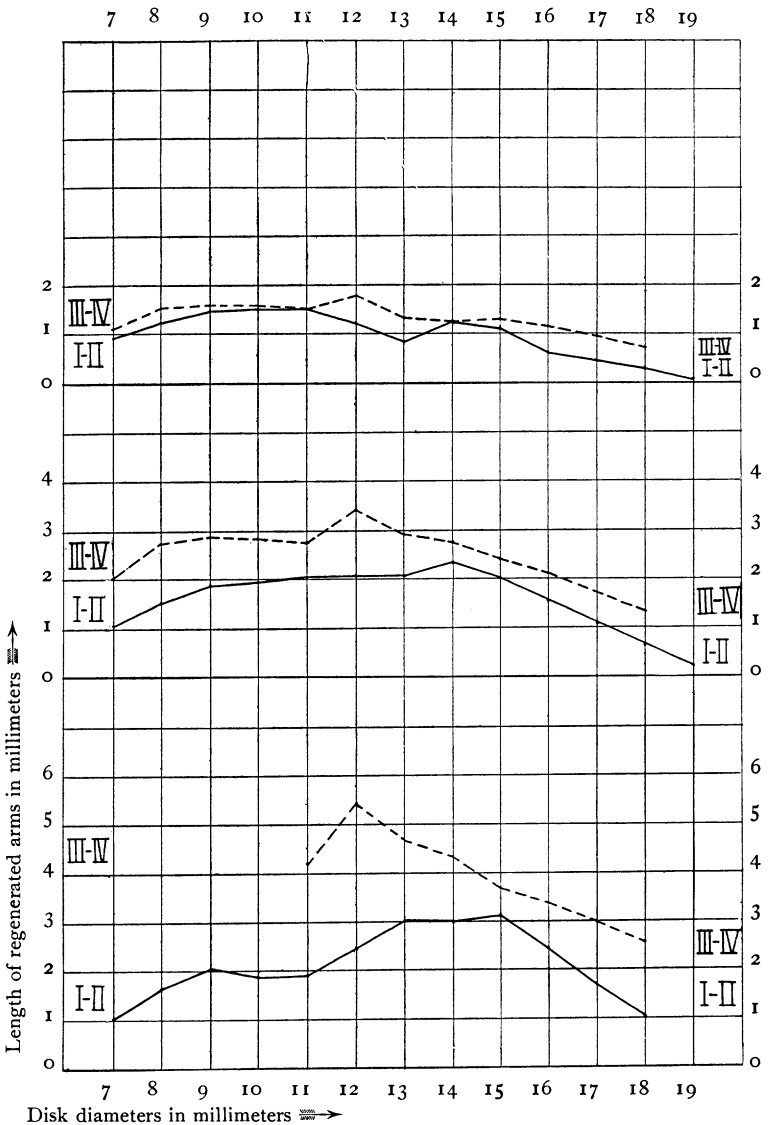

FIG. 1. The abscissæ. represent the disk diameters in millimeters and the ordinates the regenerated arm lengths, also in millimeters. The unbroken line gives the average of Series I.–II. (the specimens with one and two arms removed). The broken line gives the average of Series III.–IV. (the specimens with three and four arms removed). The uppermost curves show the conditions 22 days, the middle curves 33 days, and the lowermost curves 46 days after the operation.

urements taken 33 days and 46 days after the operation. Thus in the 33-day measurement for Series I. and II. the regenerated length increases from 1.07 mm. for a disk diameter of 7 mm. to a maximum of 2.37 mm. for a disk diameter of 14 mm. and then goes down to .21 mm. for a 19-mm. diameter. Also for the Series III. and IV. at the same time the length increases from 2.04 mm. at a diameter of 7 mm. to a maximum of 3.45 mm. at a 12-mm. diameter and down again to 1.36 mm. at a diameter of 18 mm. *The medium sized individuals thus have the maximum rate of regeneration.*

2. More striking still is the very constant difference between the average of Series I. and II. as compared with Series III. and IV. This shows a very decided advantage in favor of the animals with the greater number of removed arms. The difference is evident in the upper curves of the figure from measurements taken 22 days after the operation but becomes more striking in the 33-day and 46-day curves. For example, in the 33-day curve for a 12-mm. diameter (the diameter at which we have the maximum rate of regeneration of Series III. and IV.) we get a regenerated length of 2.08 mm. for Series I.–II. and of 3.45 mm. for Series III.–IV., an advantage of 1.37 mm. or 66 per cent. in favor of the latter. Likewise at a diameter of 14 mm. (where the Series I.–II. has its maximum regeneration) we get 2.37 mm. for Series I.–II. and 2.77 mm. for Series III.–IV., an advantage of .4 mm. or 17 per cent. in favor of Series III.–IV. In a similar manner in the curves obtained from the 46-day measurements we get at a 12-mm. disk diameter a regenerated length of 2.46 mm. for Series I.–II. and 5.42 mm. for Series III.–IV., and at a 15-mm. diameter 3.14 mm. for Series I.–II. and 3.72 mm. for Series III.–IV. which represents an advantage for the group with the greater number of removed arms of respectively 2.96 mm. (= 120 per cent.) and .58 mm. (= 18 per cent.) for the two points named.

We must therefore conclude that when more than one arm is removed the regenerative energy as expressed in the replacement of the lost arms is greatly increased. Not only is the total regenerative energy greater in this case but the energy expressed in each arm is greater than the total energy when only one is removed.

Expressing this in mathematical form, if E_1 represents the regenerative energy exhibited in the replacement of the lost arm when only one is removed, assuming that increase in length is a measure of such energy, and E_n represents the energy exhibited in regeneration when more than one arm is removed, n being the number of absent arms, then not only is $E_n > E_1$ but also $E_n/n > E_1$ or $E_n > nE_1$. Therefore when we remove n arms we increase the total regenerative energy by more than n times the amount exhibited when only one is removed. The force of this statement is made especially strong when we consider that throughout the experiments the animals received no food supply whatever.

Expressing the relation in still another way, let us take a brittle-star with arms A, B, C, D and E, in which a_1, b_1, c_1, d_1 and e_1 represent the respective lengths these arms will attain after a definite period of regeneration, supposing that one alone is cut off in each case. Now let us suppose instead that the first four are cut off, then after this same period of time we get for the regenerated lengths $a_4 > a_1$, $b_4 > b_1$, $c_4 > c_1$, $d_4 > d_1$. Now in the first case mentioned we cannot assume that the stimulus of removal and the resultant reaction of regeneration are purely local and concern only the tissues in the immediate vicinity of the cut surface for we then get into difficulty as soon as we try to explain the cases where four arms are simultaneously removed. Here we find we must add a considerable quantity (r_4) to each of the original single regeneration lengths, *e. g.*, $a_4 = a_1 + r_4$. Then $a_4 + b_4 + c_4 + d_4 = a_1 + b_1 + c_1 + d_1 + R_4$ where $R_4 (= \Sigma r_4)$ represents the total response of the organism as a whole which must be added to the local effects of the operation stimulus. If, on the other hand, we consider the influence of the organism as a whole on the regeneration of its arms as one of retardation, we must take the values a_4, b_4, c_4 and d_4 as representing most nearly the original local stimulus effect. Then without changing the values of r_4 or R_4 we may rearrange the formulæ, making $a_1 = a_4 - r_4$, etc., and $a_1 + b_1 + c_1 + d_1 = a_4 + b_4 + c_4 + d_4 - R_4$.

But whether we consider the influence of the organism as a whole to be one of acceleration or one of retardation we must recognize in either case that the regeneration rate is not a matter which involves only the local conditions at the wounded surface

as determined by the direct action of the operation. It seems, on the other hand, to be bound up with intricate reactions affecting the whole character of the activities and organization of the animal. A more direct application of the above statements to the special theories of regeneration would be out of place at the present time.

We may sum up my results on the rate of regeneration of the arms of the brittle-star, *Ophioglypha lacertosa*, as follows :

1. There is a definite relation between the size (*i. e.*, age (?)) of the animal and the rate of regeneration of its arms. The maximum rate is exhibited by individuals of medium size (with a disk diameter of 12 to 15 mm.). Both the smaller and the larger ones give a diminishing rate as we go away from this point.

2. The greater the number of removed arms (excepting the case where all are removed) the greater is the rate of regeneration of each arm.

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